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Pollen biology of ornamental ginger (*Hedychium* spp. J. Koenig)

Hamidou F. Sakhanokho[a,*], Kanniah Rajasekaran[b]

[a] USDA-ARS Southern Horticultural Laboratory, 810 Hwy 26 W, Poplarville, MS 39470, United States
[b] USDA-ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, LA 70124, United States

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ABSTRACT

An improved *in vitro* pollen germination assay was developed to assess the viability of stored *Hedychium* pollen. The effect of polyethylene glycol (PEG) (10, 15, and 20%, w/v) on pollen germination and tube growth was evaluated for *Hedychium longicornutum* and two commercial *Hedychium* cultivars, 'Orange Brush' and 'Filigree'. Overall, the inclusion of PEG 4000 in the medium improved both pollen germination and tube growth for the three different genotypes tested and the results varied depending on genotype. *In vitro* germination was used to assess the viability of *Hedychium* pollen stored up to two months. Pollen nucleus status was determined for four *Hedychium* cultivars, 'Orange Brush', 'Anne Bishop', 'Filigree', and 'Daniel Weeks'. Pollens of 'Orange Brush', 'Anne Bishop', and 'Daniel Weeks' were found to be binucleate but 'Filigree' was shown to possess both binucleate and trinucleate pollens. High pollen:ovule ratio values were obtained in several *Hedychium* taxa. The results obtained on the nuclear pollen status and pollen:ovule ratios will further our understanding of the pollination biology and help clarify the taxonomy and phylogeny of *Hedychium* species.

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1. Introduction

The genus *Hedychium* consists of about 80 species characterized by beautiful foliage as well as diverse, showy, and fragrant flowers. Some species are grown for their edible flowers and others for their medicinal or industrial properties (He, 2000; Gopanraj et al., 2005; Gao et al., 2008). Most *Hedychium* species are native to central and southeastern Asia with high concentrations in southern China and the Himalayan regions (Hayes et al., 2009). They are increasingly becoming popular as ornamental plants worldwide because of their showy and scented flowers. For example, in Great Britain where no commercial sources for *Hedychium* existed in 1982 (Schilling, 1982), the 2009 Royal Horticultural Society (RHS) Plant Finder lists 116 taxa that are available to British gardeners. Many more taxa are found in Japan and the United States (Branney, 2005). Despite the potential ornamental, medicinal, and industrial values of the *Hedychium* genus, very little work has been done to understand the pollen biology such as germinability, storage, viability, nuclear status, and pollen/ovule ratio.

In the United States, *Hedychium* plants are mostly grown in the southern part of the country where they generally flower in the summer and fall, but some species bloom in winter and spring times. This asynchronous flowering could constitute an impediment for breeders of that region to fully take advantage of the tremendous diversity that exists within *Hedychium* genus. Therefore, optimal storage conditions for *Hedychium* pollen to be used later on for pollination need to be investigated, but to assess the viability of the stored pollen, a convenient and reliable method of testing the pollen quality is required. Currently, there are no published reports available on *Hedychium* pollen storage and viability. Assessing pollen viability on the basis of its functions of delivering sperm cells to the embryo sac is not only tedious and time-consuming but also not always feasible (Heslop-Harrison et al., 1984), so other techniques including staining pollen with vital dyes and *in vitro* pollen germination and tube growth are often used to evaluate pollen viability. Furthermore, successful fertilization depends greatly not only on the ability of pollen to germinate but also on its elongation rate, assuring the successful delivery of the sperm cells to the ovules (Daher et al., 2009). *In vitro* pollen germination and pollen tube growth are greatly influenced by different factors including the composition of the germination medium, genotype of the accession, temperature and humidity. For example, addition of polyethylene glycol (PEG) to the germination medium has been reported to improve *in vitro* pollen germination frequency and tube growth by preventing tube bursting (Read et al., 1993; Shivanna and Sawhney, 1995).

Pollen of flowering plants is either binucleate or trinucleate. Compared to binucleate pollens, trinucleate pollens are known to have a rapid germination rate but short viability (Brewbaker, 1967).

* Corresponding author. Tel.: +1 601 403 8767; fax: +1 601 795 4965.
E-mail addresses: rsakhanokh@aol.com, Hamidou.Sakhanokho@ars.usda.gov (H.F. Sakhanokho).

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The generative nucleus of binucleate pollen divides in the pollen tube after germination to form two sperm nuclei while that of trinucleate pollen divides in the anther before pollen is shed (Sugiura et al., 1998). Among the 2000 species surveyed by Brewbaker (1967), (70)% were found to be binucleate and the rest trinucleate, and among the 265 families surveyed by this author, 179 had binucleate genera, 54 had trinucleate genera, and 32 had both types of genera. Furthermore, the ratios of pollen grains to number of ovules (P:O ratios) have been shown to correlate with the mating system of a plant. Cruden (1977) was the first to demonstrate that in general outcrossing species had higher P:O ratios than predominantly selfing species and conclude that outcrossed species had higher P:O ratios while selfed species had lower P:O ratios. This pollination efficiency hypothesis is based on the argument that maximum seed set in outcrossing plants would require more pollens grains as a result of inefficient pollen transfer. Pollen development and morphology are often used by taxonomists and paleobotanists to clarify the classification and identity of plant species (Fogle, 1977; Lanza et al., 1996; Mert, 2009). Ultrastructural observations under scanning electron microscopy (SEM) or transmission electron microscopy (TEM) help define pollen characteristics such as shape, size, and presence or absence of exines.

Knowledge about *in vitro* germination, tube growth, storage, nucleus status, ultrastructure of *Hedychium* pollen as well as pollen:ovule ratio can be of the utmost importance for understanding not only the basic characteristics of *Hedychium* pollen but also its pollination biology, which could ultimately lead to better breeding strategies for the *Hedychium* genus. Furthermore, it is worth mentioning that despite the attractiveness of *Hedychium* plants for industrial, medicinal, and ornamental potential, some *Hedychium* species spread so quickly that they are considered invasive or pest plants in certain regions of the world, including Australia, New Zealand, Hawaii, and Brazil (Funk, 2001; Williams et al., 2003; Soares and Barreto, 2008). Data and knowledge gained on *Hedychium* pollen through this study could contribute to the development of better control strategies for *Hedychium* species that are or could be invasive. However, information on any of the pollen characteristics listed above is either scarce or totally lacking. Therefore, the objectives of the current study are to (1) evaluate pollen germination and tube growth in different genotypes of *Hedychium*; (2) assess viability of stored *Hedychium* pollen; (3) study pollen development and biology including nucleation and ultrastructure; and (4) determine pollen:ovule ratios for selected *Hedychium* taxa.

2. Materials and methods

2.1. In vitro pollen germination and tube growth

For pollen germination test, the hanging drop technique was employed following published procedures (Loupassaki et al., 1997; Deng and Harbaugh, 2004) with some modifications as described below. A "basic" liquid medium containing 1.2 M sucrose, 0.42 g L^{-1} calcium nitrate [$Ca(NO_3)_2$], 0.20 g L^{-1} boric acid (H_3BO_3), 0.1 g L^{-1} potassium nitrate (KNO_3), and 0.22 g L^{-1} magnesium sulfate ($MgSO_4 \cdot 7H_2O$) was used. The effect of polyethylene glycol (PEG) concentrations (10, 15, and 20%, w/v) on pollen germination and tube growth was evaluated in *Hedychium longicornutum* and two other *Hedychium* commercial cultivars, 'Orange Brush' and 'Filigree'. For the germination test with the PEG-based media, both pollen germination and pollen tube growth were recorded after a 3 h incubation period. Pollen germination was determined by direct microscopic observation (at 10×) in three fields of view per slide. Four replicates (slides) were used for each plant. A pollen grain was considered to have germinated when pollen tube length equaled or exceeded the grain diameter. Germination percentage was determined by dividing the number of germinated pollen grains by the total number of pollen grains per field of view and multiplying by 100. To measure pollen tube growth, samples were prepared as described above. The slides were observed using a compound microscope (ACCU-SCOPE, Inc., Sea Cliff, NY) at 1000× oil immersion magnification. The germinated tubes were photographed with an M Eye Digital camera (Ken-A-Vision, Kansas City, MO), and tube growth was measured using the Vision Explore software (Ken-A-Vision, Kansas City, MO).

2.2. Pollen viability test

For the germination test, both pollen germination and pollen tube growth were recorded after a 3 h incubation period. For the rest of the germination test, pollen viability was evaluated after 24 h incubation. The effect of storage temperature (4 °C and −20 °C and duration (0, 4, and 8 weeks) on pollen germination was evaluated for the commercial cultivars 'Orange Brush', 'Filigree', 'Tai Golden Goddess', 'Pink V', and *H. forrestii*. Pollen germination was determined by direct microscopic observation (at 10× magnification) in three fields of view per slide. Four replicates (slides) were used for each plant. A pollen grain was considered to have germinated when pollen tube length equaled or exceeded the grain diameter. Germination percentage was determined by dividing the number of germinated pollen grains by the total number of pollen grains per field of view and multiplying by 100.

2.3. Pollen nucleus status and ultrastructure

To stain pollen nucleus, a 1% acetocarmine was prepared by diluting 1 g carmine and 0.2 g in boiling 100 ml of 45% (v/v) glacial acetic acid, which was rapidly cooled and then filtered into a dark amber glass. Pollens were collected from greenhouse grown *Hedychium* plants, transferred in 1.5 ml centrifuge tubes containing 1 (acetic acid):3 (ethanol), vortexed for about 10 s, and fixed for 24 h. Afterwards, they were centrifuged for 3 min at 1300 rpm and the liquid was poured off. Pollens were hydrolyzed in 1N HCl at 60 °C for 15 min, centrifuged for 3 min, and the acid poured off. Three to four drops of deionized water were added to the pollens, which were vortexed for 3 min before the water was removed. Thereafter, 3–4 drops of 1% acetocarmine staining solution was added to entirely cover the pollens and let set for 1–24 h. Two drops of the staining liquid containing pollens were transferred on a slide and covered with a cover slip. A bibulous filter paper was placed on top of the slide and the pollens were given a gentle squeeze to extrude pollen cytoplasm. Pollens were then examined under a light microscope (10× and 40×) to determine the nuclear number. Pollen nuclear number determination was achieved for pollens from the *Hedychium* cultivars 'Filigree', 'Orange Brush', 'Anne Bishop', and 'Daniel Weeks'.

2.4. Scanning electron microscopy

The pollen samples were collected from fresh flowers and processed for scanning microscopy without the usual fixation and dehydration procedures. The samples were directly sputter-coated with gold/palladium (200 nm) before imaging at 10–13 kV with an XL30 ESEM (FEI Instruments, Hillsboro, OR).

2.5. Pollen:ovule ratios

To count pollen grains and ovules, flowers were collected at dehiscence from seven *Hedychium* taxa, *Hedychium* cv. 'Anne Bishop', *Hedychium coronarium*, *Hedychium* cv. 'Disney', *Hedychium* cv. 'Kwense', *Hedychium* cv. 'Daniel Weeks', *Hedychium coccineum*, and *Hedychium* cv. 'Double Eagle'. Five drops of 1% acetocarmine

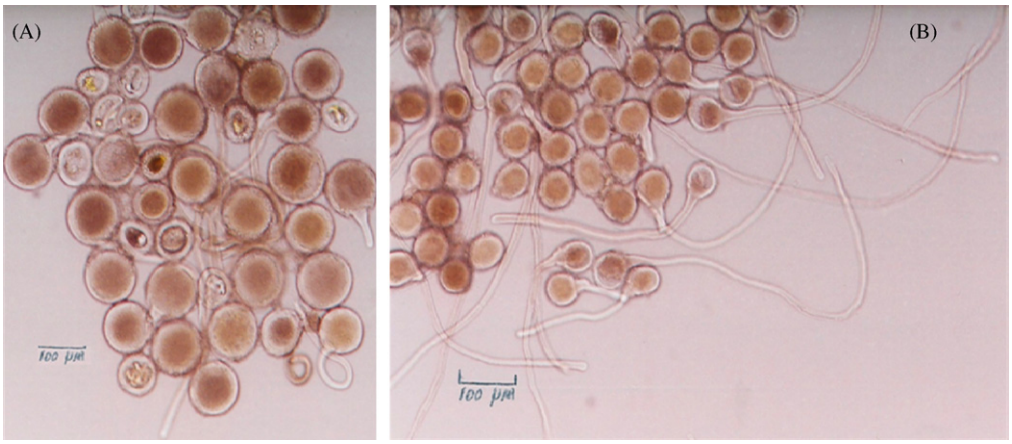

Fig. 1. Pollen germination and tube growth in (A) *Hedychium longicornutum* and (B) *Hedychium* cv. 'Orange Brush' after 3 h in a medium containing 10% PEG 4000. Magnification for A and B images = 10×.

were placed on a slide, and the anther removed from the flower and dipped in the stain on the slide to gently remove all the pollen grains. A cover glass was placed over the slide which was heated over a flask of boiling water for 30 s. The number of pollen grains was determined with the aid of a light microscope (10×). To count the ovules, the lower portion of the corolla tube was cut off and split open with a scalpel to reveal the ovary. The ovary was then placed under a light microscope, and the ovules were carefully removed with two dissecting needles, spread out, and counted. For each flower, the pollen:ovule ratio (P:O) was determined by dividing the number of pollen grains per anther by the number of ovules. For each taxon, four randomly chosen stamens and ovaries from four different plants were used.

3. Results

3.1. In vitro pollen germination and tube growth

Pollen germination and tube growth was observed for the three genotypes, *H. longicornutum*, *Hedychium* 'Orange Brush', and *Hedychium* 'Filigree' in all both the control and the PEG 4000-containig media (Figs. 1A and B and 2A and B). The effect of PEG 4000 on pollen germination and tube growth varied according to genotype (Fig. 2A and B). For *H. longicornutum*, the highest germination percentage was obtained with the control medium that contained no PEG. Actually, the germination percentage dropped for the media containing 10 and 15% PEG, respectively, before rising again to 38.0% at the 20% PEG. For 'Orange Brush', the germination percentage steadily improved with increasing PEG concentration, reaching a 24% percentage point improvement for the 20% PEG compared to the control (Fig. 2A). Among the three genotypes tested, 'Filigree' had the lowest germination percentage, which appeared to improve with the addition of 10 or 20% PEG 4000 (Fig. 2A). Both the germination percentage and pollen tube growth were recorded after a 3 h incubation period because our preliminary data had shown that after 5 h, pollen tube growth could not be measured accurately due to the extensive intertwining of the tubes. Interestingly, for *H. longicornutum*, the highest pollen tube length was obtained in medium containing 15% PEG, which had the lowest germination percentage for the same species.

3.2. Pollen viability tests

Pollen viability test via *in vitro germination* was determined for three *Hedychium* genotypes, *Hedychium forestii*, *Hedcyhium* cv. 'Tai Golden Goddess', and *Hedychium* cv. 'Pink V' (Table 1). Pollen viability estimated by *in vitro* germination varied throughout the various storage regimes for all genotypes tested. For *H. forrestii*, germination rate was highest (88.5%) for freshly processed pollen and lowest (1.4%) for pollen grains stored for two months at 4 °C (Table 1). Both storage temperature and duration affected germination rate. Compared to that of the control (88.5%), viability of pollen grains stored at 4 °C for one month decreased about 87 percentage points (Table 1). On the other hand, a high germination rate (74.8%) occurred when pollen grains were stored at −20 °C for one month. This viability dropped about 51 percentage points

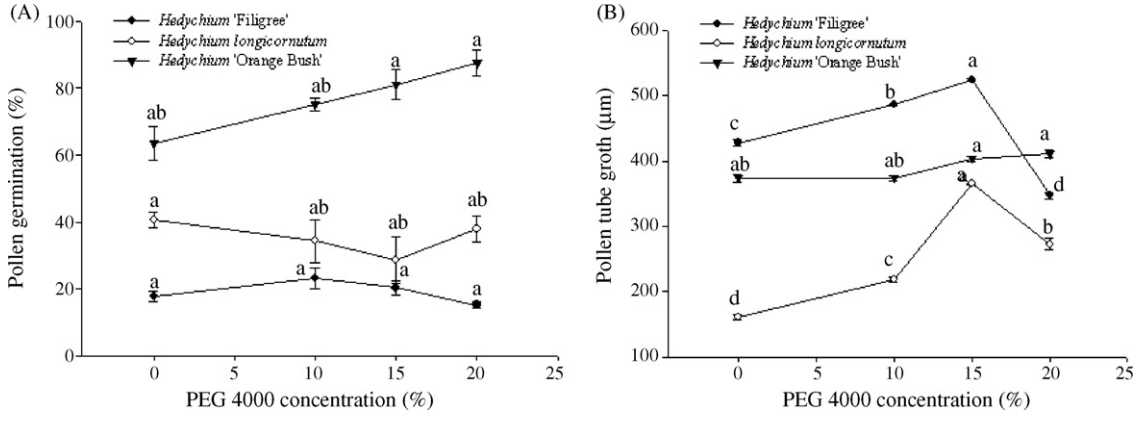

Fig. 2. Pollen germination (A) and tube growth (B) in three *Hedychium* genotypes, *Hedychium* cv. 'Filigree', *Hedychium longicornutum*, *Hedychium* cv. 'Orange Brush'. Bars with the same letter belonging to the same genotype are not significantly different ($P = 0.05$) according to Tukey's test.

Table 1
Effect of storage temperature and duration on *in vitro* pollen germination (%) of five *Hedychium* genotypes.

Genotype	Storage temperature (°C)	Storage time (week)		
		0	4	8
'Orange Brush'	Control	92.3	92.3 a	92.3 a
	4	–	32.5 c	9.6 c
	−20	–	49.4 b	28.8 b
'Filigree'	Control	20.3	20.3 a	20.3 a
	4	–	3.8 c	1.6 c
	−20	–	14.3 b	4.0 b
'Tai Golden Goddess'	Control	91.7	91.7 a	91.7 a
	4	–	6.8 c	1.6 c
	−20	–	37.2 b	24.6 b
'Pink V'	Control	83.2	83.2 a	83.2 a
	4	–	1.3 c	0.0 c
	−20	–	5.4 b	2.9 b
Hedychium forrestii	Control	88.5	88.5 a	88.5 a
	4	–	1.8 c	1.4 c
	−20	–	74.8 b	23.7 b

Means with different letters within the same column and belonging to the same genotype are significantly different ($P = 0.05$) according to Tukey's test.

(74.8–23.7%) when pollen grains were stored at the same temperature for one additional month (Table 1). For *Hedychium* cv. 'Tai Golden Goddess', 91.7% of freshly collected pollen grains germinated compared to 6.8 and 1.6% for pollen stored at 4 °C for one and two months, respectively (Table 1). However, this decline in viability was less pronounced when pollen grains of the same genotype were stored at −20 °C. At this temperature, 37.2 and 24.6% of pollen grains germinated after one and two months, respectively. For *Hedychium* cv. 'Pink V', the highest germination rate, 83.2%, occurred for freshly collected pollen, but viability dropped 77.8 (83.2–5.4%) and 80.3 (83.2–2.9%) percentage points when pollen grains were stored at −20 °C for one and two months, respectively (Table 1). When stored at 4 °C, only 1.3% of the pollen grains germinated after one month, and this viability dropped to 0% after an additional month of storage at this temperature. Over 20% of the pollens from 'Orange Brush', 'Tai Golden Goddess', and *H. forrestii* remained viable after two months when stored at −20 °C, but less than 5% of the pollens from 'Filigree' and 'Pink V' germinated when stored under the same conditions (Table 1).

3.3. Pollen nucleus status and ultrastructure

The number of pollen nuclei was determined in four cultivars, *Hedychium* cv. 'Orange Brush', *Hedychium* cv. 'Anne Bishop', *Hedychium* cv. 'Filigree', and *Hedychium* cv. 'Daniel Weeks' (Fig. 3). All examined pollens from 'Orange Brush' 'Anne Bishop', and 'Daniel Weeks' were found to be binucleate, but 'Filigree' possessed both binucleate and trinucleate pollens (Fig. 3A). An observation of 500 *Hedychium* cv. 'Filigree' pollens found 5% of the pollens to be trinucleate and the rest binucleate. Scanning electron micrograph (SEM) images of pollen grains from three *Hedychium* genotypes, *H. longicornutum*, *Hedychium* cv. 'Orange Brush', and *Hedychium* cv. 'Filigree' taken at 1200× and 2500× are shown in Fig. 4. Mature pollen grains are round (50 μM diameter) and lack elaborate exine architecture.

3.4. Pollen:ovule ratios and other characteristics of Hedychium inflorescence

Inflorescence structures and diverse flower colors of selected *Hedychium* taxa are shown in Fig. 5. The number of cincinni per thyrse, the number of flowers per cincinnus, the total number of flowers per thyrse, the number of ovules per ovary, the number of pollen grains per stamen, and the P:O ratio for *Hedychium* taxa *Hedychium* cv. 'Anne Bishop', *H. coronarium*, *Hedychium* cv. 'Disney', *Hedychium* cv. 'Kwense', *Hedychium* 'Daniel Weeks', *H. coccineum*, and *Hedychium* cv. 'Double Eagle' are shown in Table 2. The mean number of cincinni/thyrse, flowers/cincinnus, ovules/flower, pollen grains/flower, and P:O ratio/flower of the *Hedychium* taxa investigated ranged from 8.7 to 32.8, 2.2 to 4.1, 31.0 to 133.5, 4364.3 to 14244.2, and 113.7 to 239.4, respectively (Table 2). The inflorescence of *Hedychium* is a simple thyrse, consisting of a main axis bearing primary bracts that subtend lateral cincinni which can have 1–6 flowers per cincinnus (Holttum, 1950; Kirchoff, 1997). The number of flowers per cincinnus for the *Hedychium* taxa evaluated in this study ranged from 2 to 4 (Table 2).

4. Discussion

In general, the inclusion of PEG 4000 in the medium improved both pollen germination and tube growth for the three genotypes tested even though the responses varied according to PEG concen-

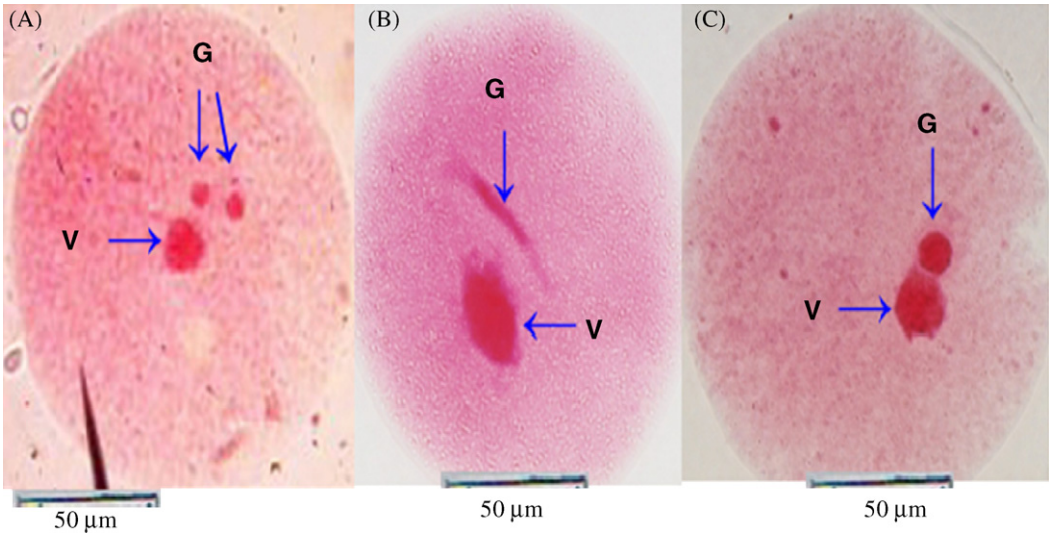

Fig. 3. (A) Mature pollen grains of *Hedychium* stained with acetocarmine to show pollen nuclei. A trinucleate pollen of cv. 'Filigree with a larger vegetative nucleus (V) and two smaller generative nuclei sperm (G) nuclei. (B) A representative binucleate pollen of *Hedychium* spp. with a vegetative (V) and a crescent-shaped generative (G) nucleus. (C) A representative binucleate pollen of *Hedychium* spp. with a larger vegetative nucleus (V) and a smaller generative nucleus (G). Magnification for A–C images = 40×.

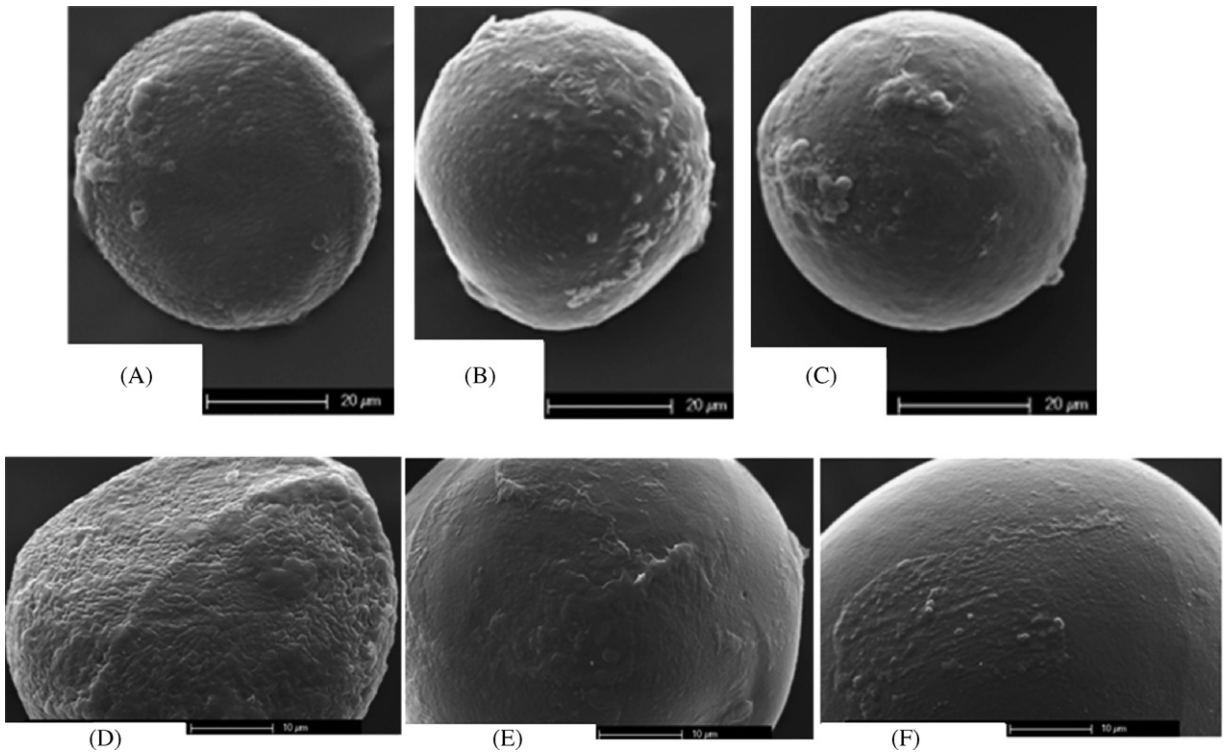

Fig. 4. Scanning electron micrographs of pollen grains from selected *Hedychium* genotypes. (A and D) *Hedychium longicornutum*; (B and E) *Hedychium* cv. 'Orange Brush'; (C and F) *Hedychium* cv. 'Filigree'. Top row (A–C) magnification = 1200×; bottom row (D–F) magnification = 2500×. These pollen grains lack a distinct exine architecture, a characteristic which has been reported in other members of Zingiberaceae.

Fig. 5. Inflorescence and flower structures in *Hedychium*. (A) *Hedychium longicornutum*; (B) *Hedychium* cv. 'Tai Pink Profusion'; (C) *Hedychium bousigonianum*; and (D) *Hedychium* cv. 'Dr. Moy'.

tration and/or genotype. For example, the germination percentage was highest for *H. longicornutum* in control medium without PEG 4000. The germination percentage dropped for the media containing 10 and 15% PEG, respectively, before rising again to 38.0% at the 20% PEG. The reason for this is unclear, but Jandurová and Pavlík (1995) reported similar results in *Brassica campestri* (but not in *Brassica napus* and *Brassica oleracea*) that showed a decrease in pollen germination with the addition of PEG 6000 after a 90 min incubation period. The peculiar response of *H. longicornutum* pollen to PEG 4000 may be due to the genetic differences in the *Hedychium* genus as it was the case for other plant species (Preuss et al., 1993; Jandurová and Pavlík, 1995; Montaner et al., 2003). The pollen tube length measurements in 'Filigree' seem to support the results obtained with *H. longicornutum* as 'Filigree' which had the lowest germination rate among all three genotypes had the highest pollen tube growth rate for 0, 10, or 15% PEG 4000 (Fig. 2B). Furthermore, the addition of PEG 4000 to the media had a beneficial effect as it prevented extrusion of pollen cytoplasm, which

Table 2
Number of cincinni/thyrse, flowers/cincinnus, ovules/flower, pollen grains/flower, and P:O ratio/flower of selected *Hedychium* taxa. For each taxon, the means (±SD) are from four different and four flowers from each plant, for a total of 16 flowers per taxon.

Taxa	Number of cincinni/thyrse	Number of flowers/cincinnus	Number of ovules/flower	Number of pollen grains/flower	P:O ratio/flower
H. 'Anne Bishop'	20.5 ± 0.3	3.2 ± 0.3	69.0 ± 1.4	13874.0 ± 385.5	201.1 ± 12.3
H. coronarium	9.3 ± 1.2	3.4 ± 1.1	133.5 ± 3.5	14210.0 ± 1989.8	106.5 ± 8.9
H. 'Disney'	32.8 ± 0.9	2.4 ± 0.5	31.0 ± 2.3	4780.5 ± 598.3	154.2 ± 102
H. 'Kwense'	15.9 ± 2.3	3.7 ± 0.9	59.5 ± 0.7	14244.2 ± 2156.3	239.4 ± 21.0
H. 'Daniel Weeks'	8.7 ± 1.2	3.1 ± 0.7	70.5 ± 0.7	8016.1 ± 763.3	113.7 ± 7.9
H. coccineum	32.1 ± 0.8	2.2 ± 1.1	33.0 ± 2.6	4364.3 ± 256.9	132.2 ± 24.5
H. 'Double Eagle'	14.3 ± 1.3	4.1 ± 0.8	55.0 ± 4.2	10144.8 ± 2003.5	185.0 ± 17.3

could lead to the bursting of germinating pollen grains after 3 h of incubation. This is most likely due to the fact that PEG is relatively inert metabolically and cannot enter cells to increase the already high internal concentrations (Subbaiah, 1983; Hoekstra et al., 1989; Kawaguchi et al., 1996). Furthermore, *Hedychium* pollen can remain viable for at least two months when stored at −20 °C even though this viability varies depending on the genotype.

Most of the pollen grains from the examined *Hedychium* taxa were found to be binucleate, but *Hedychium* cv. 'Filigree' possessed both binucleate and trinucleate pollens (Fig. 3). Though rare, the presence of both binucleate and trinucleate pollens in the same genus has been documented by some authors (Brewbaker, 1967; Hamilton and Langridge, 1976; Lora et al., 2009). The low germination percentage of *Hedychium* cv. 'Filigree' (Fig. 2A) suggests that a high percentage of the grains of this cultivar was not viable. However, the presence of both binucleate and trinucleate pollens in this genotype may help to compensate for this apparent low number of viable pollens for the following reasons. Even though the germination percentage and tube growth of binucleate and trinucleate pollens from this cultivar were not compared in this study, there is evidence that trinucleate pollens germinate and elongate at a faster (but have shorter viability) rate than binucleate pollens (Brewbaker, 1967; Mulcahy and Mulcahy, 1988; Lora et al., 2009). This faster germination rate of trinucleate pollen, which appears to rely on exogenous sources from the start of germination, can be valuable in conditions where rapid reproductive processes are needed (Mulcahy and Mulcahy, 1988; Lora et al., 2009). Trinucleate pollen is best suited for the mode of pollen dispersal that includes wind- and water-dispersal of pollen grains and slight increase in temperature, which accelerates female development and the whole reproductive process (Sanzol and Herrero, 2001). On the other hand, binucleate pollen which germinates at a slower rate but survives longer could still be available to be transferred later by pollinating insects to flowers in the female stage (Gottsberger, 1989). In the end, the presence of both binucleate and trinucleate pollens at anthesis could be viewed as a bet-hedging strategy to obtain a higher chance of fertilization (Lora et al., 2009).

Pollen morphology and ultrastructure have been shown to be correlated with growth habit and pollination biology (Osborn et al., 2001). Scanning electron micrographic images of pollen grains from selected *Hedychium* genotypes (Fig. 4) showed that these pollen grains lack elaborate exine architecture or have very thin exine. In general, pollen grains of terrestrial taxa have well defined exine, those of amphibious taxa have thinner exine, and the obligately submersed taxa have no exine (Martinsson, 1993; Osborn and Philbrick, 1994; Cooper et al., 2000). Exine reduction or loss is associated with the phylogenic framework of the family (Cooper et al., 2000; Philbrick and Les, 2000). Extremely thin and discontinuous exine has been reported in other members of the Zingiberaceae family (Theilade et al., 1993; Theilade and Theilade, 1996). Reduced exine has also been reported in other terrestrial plants (Tanaka et al., 2004). Such morphological characteristics of pollen can have implications for pollen germination, pollen viability as well as pollination mechanisms. For example, Furness and Rudall (1999) argued that an omniaperturate (a pollen grain with thin or absent exine and thick intine) condition with reduced exine might be an adaptation to increased germination efficiency because the pollen tube is formed more quickly and can emerge from any region. The role of the exine is to protect the male spore and gametophyte from desiccation and other dangers of sub-aerial dispersal (Heslop-Harrison, 1976); therefore, reduction or absence of exine in *Hedychium* pollen could adversely affect its long-term viability. Tanaka et al. (2004) demonstrated that a strong correlation between pollen morphology and pollination mechanisms in Hydrocharidaceae. These authors concluded that the selective pressures acting on the pollination mechanisms have reduced the exine structure in hypohydrophilous plants and resulted in various exine sculptures that are adapted to the different pollination mechanisms in entomophilous, anemophilous, and pollen-epihydrophilous plants. Furthermore, Wang et al. (2004) reported pollen:ovule ratios of 82.67, 138.49, and 41.85 in *H. coccineum*, *H. coronarium*, and *Hedychium spicatum*, respectively. The breeding system of all three species was classified as facultative xenogamy (Wang et al., 2004). The pollen:ovule ratio values that we obtained were generally comparable to or slightly higher than those reported by Wang et al. (2004).

5. Conclusion

We have developed an efficient *in vitro* method for pollen germination and tube growth, which could be useful for both basic and applied studies on the pollen biology of *Hedychium* species. This system could be particularly valuable in assessing the viability of stored *Hedychium* pollen because *in vitro* pollen germination is a more reliable indicator of pollen viability (Heslop-Harrison, 1979; Heslop-Harrison et al., 1984; Stone et al., 1995). In addition, to our knowledge, the results presented here on pollen nucleation represent the first report on pollen nuclear status in *Hedychium*. They indicate the presence of both binucleate and trinucleate pollens. Self-sterility is common in *Hedychium*, so species of this genus hybridize readily, which may have contributed to the current taxonomic confusion of *Hedychium* species (Wood et al., 2000; Gao et al., 2005). Very little information is presently available on the parentage of the four cultivars used in the pollen nucleation study. *Hedychium* cv. 'Orange Brush' is often listed as a hybrid in many commercial nursery websites and believed to have derived from *H. coccineum*, and *Hedychium* cv. 'Filigree' is a compact and short hybrid which is believed to have the epiphytic *Hedychium hasseltii* in its background. Both these cultivars were introduced by the nurseryman Tom Wood from Archer, Florida, USA. 'Anne Bishop' is an evergreen hybrid from Hawaii whose parentage is unknown. *Hedychium* cv. 'Daniel Weeks', introduced in 1992 by Gainesville Tree Farm of Florida, is believed to be a hybrid of *Hedychium flavescens* and *Hedychium gardnerianum* (Branney, 2005). Results obtained here on several characteristics of *Hedychium* pollen, including germination, storage and viability, nucleation, as well as pollen:ovule ratio will help better understand the pollination biology and taxonomic classification of the *Hedychium* genus.

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